1941

Inheritance and physiology of efficiency in iron utilization in soybeans

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NOTE TO USERS

INHERITANCE AND PHYSIOLOGY OF EFFICIENCY IN IRON UTILIZATION IN SOYBEANS

by

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A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subjects Genetics and Crop Breeding

Approved:

Signature was redacted for privacy.

Signature was redacted for privacy.

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Dean of Graduate College

Iowa State College
1941

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INTRODUCTION

Striking differences in chlorosis typical of iron deficiency were noted in 1938 among a considerable number of soybean varieties when tested on calcareous soils for the first time since their introduction into the United States from Manchuria. Because these strains were not greatly different morphologically, it was thought that such wide differences in chlorosis would lend themselves especially well to a study of the complex problems of differential performance of diverse plant genotypes of the same species when grown on varying nutrient media. Although interactions between genotypes and nutrient levels have been previously reported, never have such differential responses been of sufficient magnitude and definitude to permit a study of their mode of inheritance.

Since these varieties differed greatly in performance when grown in media low in available iron, they were also considered excellent material for a physiological study of iron availability in plants.

REVIEW OF LITERATURE

Extensive investigations have been reported on the differential ability of diverse plant species to grow on media of low mineral concentration, as well as on the interaction between species and added increments of nutrients. In an effort to explain these specific differences such factors in nutrient absorption as extent of root systems, carbon dioxide production, permeability of cell walls, and acidity of cell saps have been subjected to detailed investigations. Although plant nutrition is exceedingly complex, and many of these investigations may have an indirect bearing on the problem at hand, only those investigations that have a direct relation to this problem will be reviewed.

Investigations are less numerous in which differential response to varying nutrient levels has been manifested by unlike genotypes within the same species. Stringfield and Salter (40) reported that in two years out of five, corn varieties and hybrids interacted significantly with four fertility levels when grown in a corn, oats and wheat rotation. In this same rotation Lamb and Salter (21) found that 11 wheat varieties did not respond differentially to a significant degree in yield of grain on the four fertility levels in any one year, but when the data for five years were combined, a significant "varieties x fertility levels" interaction was obtained. The differential response to fertility levels manifested by oat varieties in this same rotation was not found to be significant (22). Seed yields of eight soybean varieties when grown for three continuous years on these fertility levels showed a non-significant

"varieties x levels" interaction (32). Neither was the interaction for percent protein, percent oil and size of seed significant. Varieties did, however, interact significantly with fertility levels in iodine number of oil. This interaction was attributed to slight differences in time of maturity on the four fertility levels, which thereby allowed the fatty acids to be metabolized in slightly different temperature environments.

Differential growth on clay and loam soils was exhibited by different inbreds and hybrids of corn according to Hoffer (14). Leaf tissue analyses indicated heritable differences between the selfed lines relative to absorption of phosphorus, iron and aluminum from the soil solution. Gregory and Crowther (10) and DeTurk, Holbert and Hawk (7) working with barley and corn respectively, found differential response of diverse genotypes to various fertilizer treatments.

Striking differential efficiency was found by Smith (38) between inbred lines of corn when grown on low phosphorous media. Performance of hybrids between efficient and inefficient lines indicated dominance of the efficient type. Hybrids among inefficient lines were inefficient in performance. Efficiency in phosphorous utilization seemed correlated with a high proportion of secondary to primary roots. The results obtained by Lyness (25) with corn inbreds and hybrids are in agreement with Smith's findings.

Differential ability of inbred lines of corn to utilize nitrogen in the ammonium form was demonstrated by Harvey (12). Partial dominance of the ammonium nitrogen utilizing ability was indicated by the performance of hybrids between the two types. In media varying in nitrogen content,

Burkholder and McVeigh (4) found differential growth of inbred lines and hybrids of corn particularly noticeable at the higher increments of nitrogen.

Results from certain genetic investigations of an entirely different nature seem to have a bearing on the ensuing report. Genetically controlled differences in the acidity of flower petal sap of Primula sinensis, P. acaulis, Papaver Rhoeas and other species have been reported by Scott-Monorieff (35, 36). In all species studied the differential pH of petal sap was shown to be due to a single gene difference. When petals of P. sinensis plants homozygous for the recessive gene causing low acidity were macerated and diluted with water, the mixture showed a pH of approximately 6.0. Presence of the dominant allel resulted in a flower petal pH of approximately 5.3. The characteristic conditioned by the gene seemed to consist of a single localized specific increase in the acidity of the petals. Expression of this characteristic was suppressed by certain anthocyanin inhibiting genes.

The important role of iron in chlorophyl formation was appreciated as early as 1844 (11). Early in the 20th century Willstätter and Stoll (44) discovered that magnesium was the metal in the chlorophyl molecule, and although iron was not a constituent of this molecule, it was shown to be necessary for chlorophyl formation. Although numerous investigations have been conducted on the availability of iron for plants since that date, the problem is still unsolved in several aspects.

Reaction of the growth medium as it affects availability of iron to plants has received considerable attention (1, 2, 9, 17, 23, 26, 27). Severe chlorosis of plants grown on soils containing an excess of lime

has frequently been reported. Most of the early investigations assumed that the high $CaCO_3$ concentration or the associated low hydrogen ion concentration in the soil caused iron to be precipitated, thereby rendering it non-available to plants. In nutrient solution studies, lack of normal chlorophyl development due to iron deficiency also has frequently been encountered. As solutions with high pH provoked chlorotic symptoms more readily than solutions with low pH, and as the precipitation of iron as the hydroxide in alkaline solutions was recognized, the problem again was considered entirely one of availability of iron to the roots.

The precipitation of iron in solutions of different levels of hydrogen ion concentration has consequently received considerable attention. Patten and Mains (31) reported that when ammonium or sodium hydroxide was added to a dilute solution of ferric chloride, precipitation of ferric hydroxide was first noticed at a pH of 3.5 as a faint cloudiness, and increased in degree until at a pH of 6.0 a very heavy precipitate resulted. Johnson (19) determined the titration curve of ferric chloride when titrated with sodium hydroxide. His results indicated a complete precipitation of the iron at pH 4.4. After filtering this solution, analysis of the filtrate showed no trace of iron. The titration curve for ferrous sulphate was also determined, and analysis of the solution indicated a strong test for soluble ferrous iron until a pH of nearly 9 was attained. As investigations cited later assume the precipitation of iron as ferric phosphate, it may be well to summarize the investigations of Teakle (41). When mixtures of NaH_2PO_4 and $FeCl_3$ were

adjusted to various hydrogen ion concentrations, ferric phosphate was found to be least soluble under acid conditions corresponding to pH 3. Under conditions of lower pH, the ferric phosphate was more soluble, while with less acid conditions, ferric hydroxide was formed at the expense of ferric phosphate thereby releasing the phosphate iron.

Tottingham and Rankin (43) and others found that when iron was added to nutrient solutions in the form of organic compounds such as ferric citrate, plants did not show symptoms of iron deficiency as quickly as when it was added in an inorganic form. Although there is little agreement among research workers as to the reason for iron being more available in organic compounds under conditions of high pH, Clark (6) and others suggested that if the principle involved were known, it might also explain why the pH at which iron deficiency symptoms are apparent is much higher in soils than in nutrient solutions.

Hopkins and Wann (17) also found the use of ferric citrate effective in maintaining available iron in the solution, but in all solutions more alkaline than pH 5.7, the iron supply was still inadequate for the growth of Chlorella. This was attributed to the adsorption of the iron on the amorphous precipitate of calcium phosphate formed in solutions at this pH. In nutrient solutions free of calcium, an adequate supply of iron could be maintained in solutions as alkaline as pH 7.5.

Discoveries by Hendrickson (13), Hoffer and Carr (15), Loehwing (24), Milad (28) and others have somewhat altered the concepts of iron availability. Their investigations have shown that chlorotic tissues in many cases contain as much or more iron than normal tissues. As iron may occur in abundance in tissue of chlorotic plants, it would seem that much

or all of this iron is in non-available form, which suggests the possibility that some internal factors may be involved in its availability to chlorophyl containing cells.

Investigations carried on by Olsen (29) may have a bearing on this point. When Zea mays, Lemna polyrhiza and Xanthium spinosum were grown in Knop's nutrient solution maintained at various hydrogen ion concentrations and using ferric chloride as the source of iron, the dry weight of plants gave a bi-modal curve when plotted against pH of the growth medium. Plants growing in solutions held at a pH of 4.5 or 8 showed no chlorosis but those growing in solutions of pH 6 or 7 exhibited severe chlorosis. Analysis of the nutrient solutions showed decreasing concentrations of soluble iron with increasing pH until a minimum was encountered at pH 6. Thereafter only small amounts of iron remained in the solution in a soluble form. Analysis of the leaf tissues of maize revealed a larger amount of iron per unit weight of dry matter in chlorotic plants (pH 6 and 7) than in normal plants. The phosphate, calcium, magnesium, potassium and nitrogen content of chlorotic leaves was also higher. Olsen surmised that this was probably due to the fact that, "... when chlorosis appears the production of organic matter will cease while the absorption of salts from the nutrient solution continues. The abnormally high amounts of inorganic substances which the chlorotic plants contain are thus a consequence of the chlorosis, and not its cause."

The conclusion was drawn that although adequate iron was absorbed in all cultures, at pH 6 and 7 considerable phosphate ions were also absorbed, resulting in precipitation of ferric phosphate within the vascular bundles of the corn plants. At pH 8 a large portion of the phosphate

was precipitated in the solution as calcium phosphate, thereby rendering phosphate concentration low in the tracheal sap, resulting in adequate availability of iron. Olsen contended that at pH 4 and 5, corn plants normally absorb less phosphate than at a higher pH, and, furthermore, as numerous investigations have shown that the pH of the growth medium influences the pH of the cell sap to some extent, the tracheal sap was adequately high in hydrogen ion concentration to prevent precipitation of iron.

These conclusions were supported by later work. The experiment with corn was repeated using one-fifth, normal and four times the phosphate concentration previously employed. With the low phosphate solution, no chlorosis occurred in plants grown in media varying from pH 4 to 8. Chlorosis again occurred at pH 6 and 7 at the normal phosphate concentration. With the abnormally high phosphate solutions, chlorosis occurred at pH 6, 7, and 8. Olsen surmised that in the latter case phosphate was added in excess of the calcium in the solution and a considerable amount remained in the solution after all calcium was precipitated. This viewpoint was supported by the low calcium content of plants grown at pH 8 with a high level of phosphate.

Olsen believed the precipitation of ferric phosphate in the vascular bundles probably occurs to a great extent only in plants grown in nutrient solution media, as the phosphate concentration of normal soil solutions is not sufficiently high to permit this precipitation.

Kliman (20) postulated that iron entered the plant largely as the ferrous cation combined with the bicarbonate or other acid radicals. It was transported within the plant in the ferrous state and was utilized

by plants in this form only. Prior to absorption by the plant, iron in the ferric state was reduced through the action of soil organic matter, microorganisms, or reducing substances in the epidermis of the plant roots. Iron accumulated within the plant in the ferrous state with the exception that in older plants some accumulations of ferric iron occurred. Within the regions of use the ferrous cation in combination with certain proteins formed a complex anion. In soils containing plant residues this complex anion was broken down by microorganisms thereby releasing the ferrous cation. In the anion form, iron remained in solution even under strongly alkaline conditions but was not absorbed by plants in this form. Kliman states that the test for ferrous iron in the leaves of dicotyledonous plants was less distinct than in the case of monocotyledonous plants, and sometimes was not obtained at all in the former case.

Iron availability to chlorotic corn seedlings was determined by Kliman in dilute solutions of ferric chloride and ammonium sulphate, monocalcium phosphate and peat extract. Recovery of the corn seedling in a check solution of dilute ferrous sulphate preceded recovery in any of the above solutions. Recovery in the ammonium sulphate-ferric chloride solution occurred within a few days, in the peat extract-ferric chloride solution only after considerable microorganic activity, but not in the phosphate-ferric chloride even after four weeks. Kliman concluded that the iron formed a complex ion with the sulphate and phosphate radicals and with acids in the peat extract, and, therefore, was not immediately available to the plants. The complex ion formed with the phosphate radical seemed quite non-available.

Additional pertinent work on the availability of iron within plants has been done by Shive and his collaborators. Ingalls and Shive (18) in studying diurnal and noctural variation in pH of expressed tissue fluid found that not only did the hydrogen ion concentration fluctuate over a 24-hour day and night period, but a high correlation existed between the hydrogen ion concentration and the soluble iron content of the fluid. Furthermore, species of plants in which the expressed tissue fluid was normally low in pH were relatively low in total iron content and a large portion of this iron was in a soluble form, whereas species with a normally high pH of the cell sap contained a much greater amount of iron within the plant and as low as four percent of it was in soluble form.

In continuing this work Rogers and Shive (33), when investigating two species, Oxalis repens and Rumex acetosella, with extremely low pH of the expressed tissue fluid, found that similar fluctuations of pH occurred throughout a 24-hour period. In the case of Rumex, a direct correlation was found between the hydrogen ion and the soluble iron concentrations of the tissue fluid even though the highest value obtained approached pH 2.7, considerably below the precipitation point of ferric hydroxide according to Patten and Mains (31). This relationship was not found in the Oxalis species. Tissue fluid of Soya max and Solanum tuberosum, species with high pH tissue fluid, also varied proportionately in hydrogen ion and soluble iron content throughout the 24-hour period even though, in the case of Solanum, pH of the fluid was above the point specified by Patten and Mains at which all iron is precipitated. Rogers

and Shive concluded that the range over which iron precipitates within the plant was greater than that found in organic solutions.

A microchemical examination of hydrogen ion concentration and iron distribution of sectional areas of plant tissues was made by Rogers and Shive. By colorimetric methods the following approximate pH values of various tissues of the soybean stem were obtained:

Tissues	pH
Xylem (vessels)	5.0-4.6
" (parenchyma)	6.6-6.2
Sclerenchyma	5.0-4.6
Phloem	7.4-6.8
Cortex	7.0-6.6
Pith	7.2-6.6

Regions of relatively low pH alternated with regions of high pH outward from the xylem. This was reflected in the distribution of total iron within the tissues. While iron occurred generally throughout the xylem tissues, it accumulated to a marked degree in the border regions of the phloem (high pH) adjacent to the xylem and sclerenchyma tissues (low pH) located on either side. In all species of plants studied small amounts of iron occurred generally throughout regions of low pH and accumulated in regions of high pH adjacent to those of low pH tissues. Accumulations of iron were assumed to consist of precipitated iron which was non-available to the plant. Species with relatively low pH of cell sap contained no iron accumulations.

Investigations conducted by Loehwing (24) with wheat, oats and corn when grown on various muck soils may have a bearing on the investiga-

tions reported here. Addition of 0.4 percent calcium carbonate to the soil resulted in copious precipitation of iron in the roots and lower stem regions of plants growing therein. Leaves of these chlorotic plants were very low in iron content. This was attributed to the fact that the calcium carbonate treatment consistently increased the pH of the plant sap extract, thereby causing immobility of the iron immediately upon entering the plant. Addition of 0.04 percent potassium chloride to the soil increased the iron content of the leaves and prevented chlorotic symptoms of iron deficiency. Furthermore, associated with the addition of potassium was noted a consistent decrease of pH in the expressed plant sap. Plants growing in this culture also were low in calcium and magnesium content to the extent that in soils originally low in calcium or magnesium, deficiency symptoms of these elements were exhibited by the plants. Contrariwise, addition of calcium carbonate decreased the absorption of potassium - to the point of starvation in some cases - indicating an interaction of calcium and magnesium with potassium.

METHODS OF PROCEDURE

Source of Genetic Material

From approximately 20 soybean varieties which exhibited iron deficiency symptoms when grown in 1938 on an experimental field in which the soil contained from 5 to 30 percent calcium carbonate, six varieties were selected for the following studies. Although varieties were selected which exhibited chlorosis most severely, it is to be emphasized that, because of the extreme soil heterogeneity in calcium carbonate content, these varieties may not be genetically more inefficient in iron utilization than many of the other chlorotic varieties. The varieties were introduced from the Orient by the Division of Foreign Plant Introduction, United States Department of Agriculture, and are known only by their Foreign Plant Introduction number. Pure strains of four standard varieties commonly grown throughout the corn belt were chosen as efficient varieties for this study. These varieties usually exhibited chlorosis only on soils containing above 40 percent calcium carbonate.

Crosses were made reciprocally in all possible combinations between the four efficient and six inefficient varieties during the summer of 1939. Because of limited greenhouse facilities F_1 plants of only two efficient varieties crossed in all combinations with four inefficient varieties were grown the subsequent winter. F_2 populations of these crosses were grown during the summer of 1940 as well as the F_1 generation of all crosses obtained the previous summer. In 1940 crosses between the

efficient and inefficient varieties were remade, as well as all possible crosses within the efficient and inefficient parents. Approximately 300 seeds also were obtained of inefficient x efficient F_1 plants back-crossed to the inefficient parents. Material available for inheritance studies during the winter of 1940-41 therefore consisted of (1) F_1 seedlings of all possible crosses among the six inefficient parents, (2) F_1 seedings of all possible crosses among the four efficient parents, (3) F_1 and F_2 seedlings of all possible crosses between the efficient and inefficient parents, (4) F3 lines of all possible crosses between two efficient and four inefficient parents and (5) a limited amount of seed resulting from the backcross of inefficient x efficient F_1 plants to the inefficient parents.

Media for Genotype Classification

The major problem in the study of the inheritance of varietal differences in efficiency was to obtain a suitable growth medium in which the proper concentration of iron could be maintained to give the differential response exhibited on certain calcareous soils. To enable accurate classification of segregating progenies, the medium should be completely homogeneous, available in large quantities to permit growing a sizeable population at one time under identical conditions, and permit differentiation between efficient and inefficient plants as early in the life of the plant as possible.

Classification of efficiency types was not found feasible in highly calcareous soils in the greenhouse. As the deposition of calcium

carbonate in the high lime soils of Iowa is very non-uniform, no source of adequately uniform soil was available and no degree of mixing of this heterogeneous soil rendered it homogeneous in available iron. Under conditions of low light intensity encountered throughout the winter months, variation of chlorosis within identical genotypes during the development of the first and second trifoliate leaves of the plants was of sufficient magnitude to render this medium unsuitable for classification of segregating progenies over a period of several months. Under conditions of rapid growth conditioned by high light intensities, especially if the plants were allowed to reach the blossoming stage before classification, thoroughly mixed calcareous soil was found suitable.

Another variable, introduced when soil was used as a growth medium, was the moisture content of the soil. Inefficient, chlorotic plants were found to develop normal green leaves when the soil was saturated with water for any length of time. Similar results were reported with rice in submerged soil by Gile and Carrero (9) which Johnson (19) concluded was due to the fact that, as reducing conditions prevailed in such soils, ferrous iron was formed which remained available to plants in alkaline media. Soil moisture uniformity, therefore is important, and maintaining such uniformity is nearly impossible at any point short of saturation.

The use of quartz gravel as a differential growth medium was attempted. Neither partial removal with acids of the iron naturally present in crushed quartz nor complete removal with the subsequent addition of magnetite, found to be a suitable source of iron by Chapman (5), proved to be suitable. Under the prevailing conditions of low light

intensity, clear cut differentiation between different genotypes was not obtainable early in the life of the plant.

In an effort to obtain a homogeneous growth medium, nutrient solutions were tried. Although uniform growth conditions could thereby be obtained, adequate amounts of available iron could not be maintained in full nutrient solutions for normal growth of even the efficient varieties without maintaining the solution at a pH well below 6. As nutrient absorption causes the pH of a solution to fluctuate greatly, this method was considered impractical.

When phosphorus was restricted in the nutrient solution, however, as previously shown by Olsen (29), no iron deficiency symptoms occurred even though the pH of the nutrient solution remained at approximately 7.4. Furthermore, it was found that when an excess of iron tartrate was added to the solution, phosphate could be added in increasing amounts until the concentration of available iron was such that the iron efficient varieties were normal in every respect while the inefficient varieties exhibited severe chlorosis at the first trifoliate leaf stage. To maintain this differentiating level of available iron it was necessary to add small quantities of phosphate to the solution every few days. If no additional phosphate was added, the chlorotic plants would slowly develop chlorophyl, presumably because absorption of the phosphate in the solution would render additional iron available. As the differentiating level could be maintained readily, this media was chosen for classification of genotypes.

Two large tanks were constructed in which approximately 1400 seedlings could be tested at one time. The tanks were constructed with wood thoroughly coated with tar-free, low penetration asphalt. They were

fitted with asphalt emulsion-coated plywood covers in which holes were drilled to permit suspension of the seedlings. Smith's (38) nutrient solution was used minus the KH_2PO_4. Approximately one part per million of iron was added in the form of ferric tartrate. An initial addition of slightly more than one part per million of the phosphate ion added as KH_2PO_4 or $Ca(H_2PO_4)_2$ established the desired differentiating level of available iron. The solution was replenished with approximately one-fifth part per million of phosphate every two days to maintain the desired level of available iron.

Classification of Genetic Material

All of the seed for the F_1, F_2, F_3, and backcrosses was germinated in gravel and, after emergence, the seedlings were transferred to the nutrient solution tanks. Parent plants were always grown with the various populations and the extent of differentiation in chlorosis among the parents was used as a criterion for establishing the appropriate level of available iron. All material was inspected at least three times at two-day intervals and classified according to iron deficiency symptoms as evidenced by chlorosis. Each plant was assigned a score ranging from 0 to 5 based on the following degree of chlorosis symptoms displayed by the youngest leaf:

0 - Dark green

1 - Light green but no definite symptom of iron deficiency

2 - Light green to slightly yellow and showing a very slight degree of chlorosis characteristic of iron deficiency

3 - Moderately yellow and showing definite iron deficiency chlorosis as evidenced by dark green veins and yellow interveinular tissue

4 - Definitely yellow with only a few of the largest veins exhibiting chlorophyl

5 - Varying from a bleached yellow appearance to complete necrosis of the leaf and death of the growing point.

Efficient and inefficient parent plants were found to display the most distinct differentiation slightly before the first or second trifoliate leaf attained maximum size. After this stage, even if chlorosis was adequately severe to develop partial necrosis, some chlorophyl would be developed. As all plants were not in exactly the same stage of leaf development at any one time, several readings were made to determine the maximum grade. During the winter months with limited light intensity and subsequent slow growth, chlorosis of the inefficient types was not sufficiently severe to prevent development of the second trifoliate leaf. Throughout the spring months, the more favorable conditions for rapid growth caused iron deficiency to be more apparent in the inefficient genotypes, and these plants seldom developed beyond the first trifoliate leaf stage before death of the undeveloped second trifoliate leaf and growing point occurred.

Sampling for Compositional Differences Between Parents

In the study of compositional differences between the parents, it was considered advisable to grow these varieties under conditions which

would promote optimum growth for both types. Seed of the four efficient and six inefficient parental varieties was germinated in gravel and, after emergence, the seedlings were suspended in a nutrient solution tank. Four plants of each variety and four replications were included. Smith's (38) full nutrient solution was used with the omission of KH_2PO_4. Approximately one ppm. of iron was added as iron tartrate and approximately 0.7 ppm. of phosphate as $Ca(H_2PO_4)_2$ was supplied several days later. A moderate degree of chlorosis was allowed to develop in the first trifoliate leaf of the inefficient varieties to prove their genetic purity. Approximately 4 ppm. of iron in the tartrate form was then supplied whereupon all plants developed normal chlorophyl and subsequent leaves showed no iron deficiency symptoms. Phosphate was added frequently in very small quantities and it was presumed an adequate amount was available as no plants exhibited nutrient deficiencies. Daylight was supplemented with artificial light from Mazda bulbs so that the plants were subjected to a 17-hour period of illumination. Under these conditions no flower bud formation was observed.

When the plants attained a height of approximately 14 inches, at which time they possessed an average of six trifoliate leaves, two replications were removed from the tank. Immediately upon removal the four plants of each variety were separated into two portions consisting of stems and leaves. The leaves were rapidly divided into two samples by cutting each leaflet longitudinally with nickel plated shears. Stems were cut into one-half-inch lengths, mixed thoroughly and divided into two samples. One of the two leaf and stem samples was immediately placed in a small, tightly stoppered vial, and dropped into a small chamber di-

rectly on a layer of dry ice. The other sample was placed in a drying chamber at 125° C.

The frozen leaf and stem tissue samples were removed to a -20° F. cold chamber until all samples were collected. Several hours later each sample was removed as needed, immersed in tepid water for several minutes, removed from the vials and placed in a small plant press constructed of low-iron brass. This press was inserted in a large vice and the composite tissue fluid extracted. The fluid was caught in a glass vial and all or a portion of it, depending on the amount obtained, was transferred to the small cup of a glass electrode attachment to a Coleman 3C pH electrometer suitable for making determinations on micro-quantities of liquids. The pH of the fluid was subsequently determined, the fluid poured back into the vial and passed through a filter paper. One ml. of the filtered fluid, or a known portion of one ml. when that quantity was not available, was then pippetted into a pyrex test tube for subsequent determination of soluble iron.

The other tissue sample, which was allowed to dry, was ground with a mortar and pestle, again placed in the drying chamber for several days, and stored in a desiccator until total iron determinations were made.

The remaining two replications in the nutrient solution tank were harvested two weeks later and prepared for analysis as previously described.

Analytical Procedures

Iron analyses were made using the o-phenanthroline method as described by Saywell and Cunningham (34). Deviations from their technique consisted only in the choice of acids for wet ashing and in the method of determining color intensity. The size of sample for total iron determinations of ground leaf and stem tissue was approximately 0.05 gm. Soluble iron was determined on the one ml. (or portion thereof) sample of filtered tissue fluid. In either case one ml. concentrated H_2SO_4 and one-half ml. concentrated HNO_3 were used for digestion of organic matter. All excess HNO_3 was removed by boiling. Reduction of the ferric iron was accomplished with an excess of hydroxylamine-hydrochloride. One ml. 0.10 percent aqueous solution of o-phenanthroline was subsequently added. The sample was then diluted with two ml. of distilled water to diminish the violence of the subsequent neutralization with concentrated NH_4OH, Congo red paper being used as an indicator. Thereafter the solution was made up to 10 ml. volume in the case of the stem samples and 50 ml. volume in the case of the leaf samples.

Intensity of the red ferrous o-phenanthroline complex was measured with an Evelyn photo-electric colorimeter. Since Fortune and Mellon (8) found the maximum absorption band of the ferrous o-phenanthroline complex to be near 7080 angstrom units, a filter with a transmission maximum of 7200 angstrom units was used. The absorption curve was determined by galvinometer readings for standard iron solutions of various dilutions when plotted against parts per million of iron in a 10 ml.

volume. This enabled transposition of galvinometer readings of samples with unknown iron content to parts per million of iron in 10 ml. volume. The mg. of iron per gm. of dry matter, in the case of total iron, and ppm. of composite tissue fluid was subsequently calculated.

The ground, dried leaf tissue was analyzed for total potassium. The procedure employed was the modified sodium cobaltinitrite method as described by Brown, Robinson, and Browning (3). Sample size and method of digestion were the same as used for the determination of total iron. The percent of potassium was calculated on a dry weight basis.

RESULTS

Parental Differentiation on Calcareous Soil

The six inefficient and the four efficient varieties in regard to iron utilization did not differ greatly as may be noted in Table 1. Dates of flowering represent the mean of three, and maturity dates the mean of two year's data. The varieties are assigned key numbers for reference in subsequent tables or discussion.

Table 1. Variety name or introduction number, assigned key number, color of flowers and pubescence, and dates of flowering and maturity of four efficient and six inefficient soybean varieties in regard to iron utilization.

Variety	Assigned Key No.	Flower Color	Pubescence Color	Date of Flowering*	Date of Maturity
Efficient:					
Dunfield	1	White	Gray	7-13	9-26
Mandell	2	Purple	Tawny	7-7	9-22
Illini	3	White	Gray	7-13	9-29
Mukden	4	White	Gray	7-8	9-19
Inefficient:					
PI 54619-5-1	5	White	Gray	7-8	9-22
PI 88508	6	White	Gray	7-7	9-18
PI 88358	7	White	Gray	7-9	9-28
PI [illegible]	8	White	Gray	7-8	9-19
PI 87617	9	Purple	Gray	7-7	9-20
PI 88354	0	White	Gray	7-7	9-26

*Date when 50 percent of plants had flowers.

Typical differential response of efficient and inefficient varieties when grown in low iron media is shown in Fig. 1.

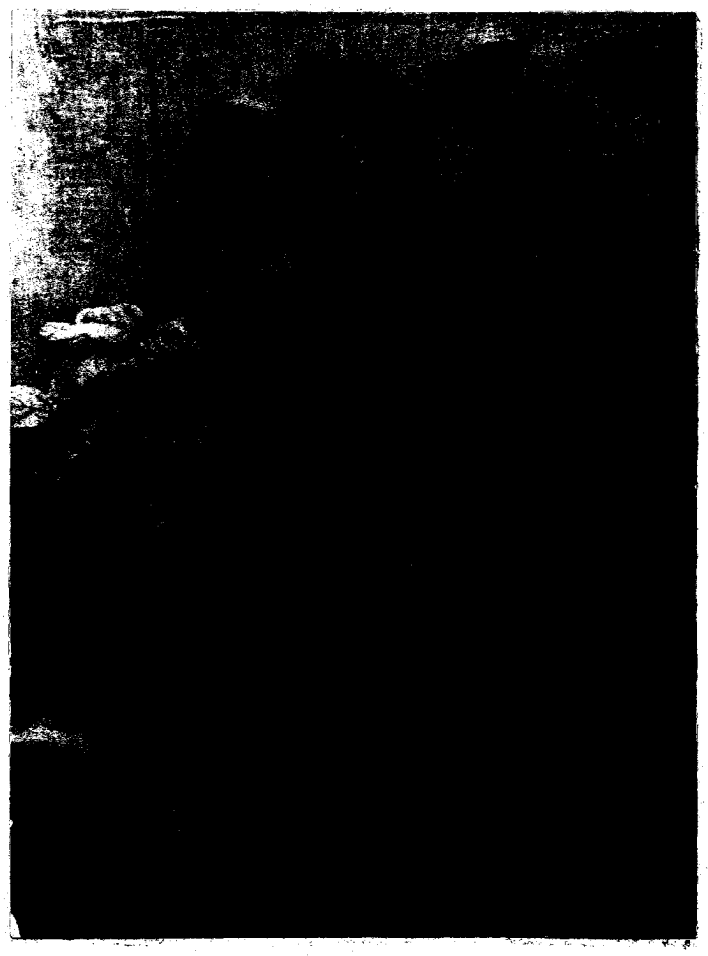

Fig. 1. Typical differential response of efficient (right) and inefficient varieties of soybeans when grown in low iron media.

The plants shown(Varieties 6 and 4) had been grown in crushed quartz from which part of the naturally occurring iron had been removed by dilute acid treatment. The quartz was sub-irrigated once a day with a nutrient solution containing adequate quantities of all elements needed for normal growth except iron. Under these conditions, and with the limited light intensity during the winter months, the growing point of

inefficient genotypes usually remained alive, but extremely chlorotic leaves were formed. Severe chlorosis frequently did not occur in the inefficient genotypes until after development of the second trifoliate leaf. This was also true when soil high in calcium carbonate and, consequently , low in available iron, was used.

Under light intensities adequate for normal growth, as in summer, chlorotic symptoms typical of iron deficiency occurred much earlier in the development of the genetically inefficient plants. To test the magnitude of the differential performance of the genetically efficient and inefficient types, four seedlings of each of the ten varieties were transplanted into each of two pots; one contained normal soil and the other was filled with soil obtained from a naturally occurring calcareous area containing approximately 30 percent calcium carbonate and having a pH of approximately 7.3. Three replications of the ten varieties grown in the two soils were transplanted on April 3, 1941. Excellent light intensities prevailed, and extreme chlorosis occurred in the inefficient plants when grown on the high carbonate soil, while no deficiency symptoms occurred in the efficient genotypes. One replication of the ten varieties growing in the high carbonate soil 14 days after the seedlings were transplanted is shown in Fig. 2.

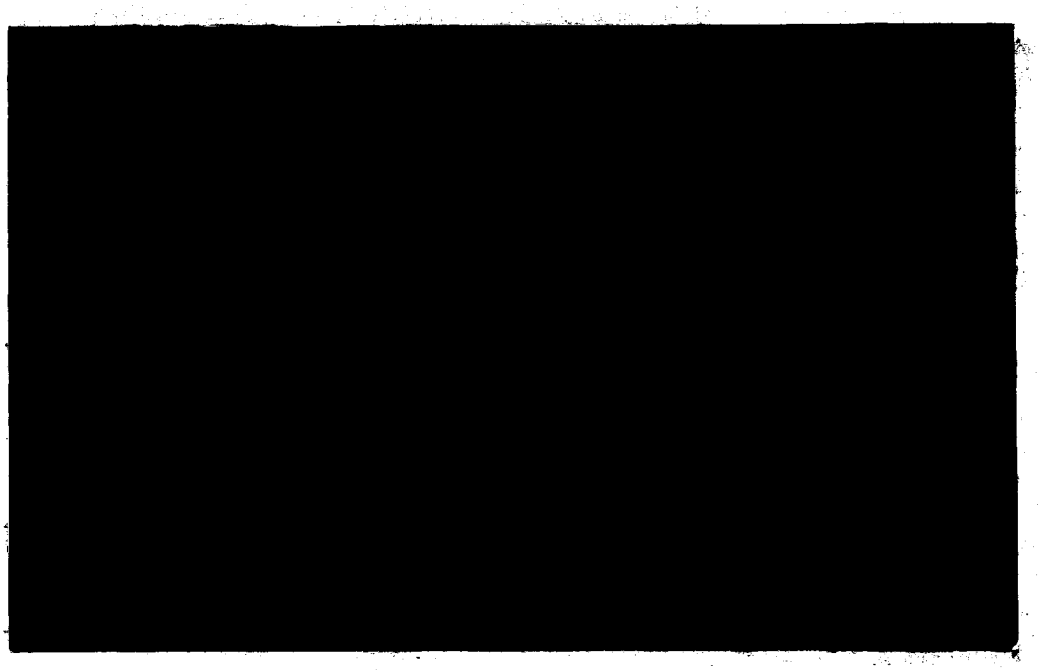

Fig. 2. Four iron efficient (left) and six inefficient (right) soybean varieties 14 days after transplanting of seedlings into calcareous soil in which available iron was low.

The extreme chlorosis of the inefficient plants is clearly demonstrated in this picture. Although the first simple ovate leaves of the inefficient soybean seedlings seldom exhibited chlorosis, the first compound leaf became severely chlorotic when available iron in the growth medium was low, and when adequate light intensities existed for maximum growth. The differential growth of the efficient and inefficient genotypes is already clearly discernible in Fig. 1.

The experiment was terminated on April 29. At this time no difference between the efficient and inefficient varieties, as judged by growth of tops, was observed in the normal soil with adequate iron (Fig. 3). Pronounced differences were discernible in the high carbonate soil in which available iron was limited (Fig. 4).

Fig. 3. Four efficient (left four pots) and six inefficient (right six pots) soybean varieties 26 days after transplanting into soil with adequate available iron.

Fig. 4. Four efficient (left four pots) and six inefficient (right six pots) soybean varieties 26 days after transplanting into soil with low available iron.

The four plants in each pot were harvested as a unit by severing the stems at the surface of the soil. They were weighed and placed in a drying chamber. Mean dry weights from three replications are presented in Table 2 for the ten varieties grown in the two soils.

Table 2. Mean dry weight in grams from three replications of four efficient and six inefficient varieties grown in soils with adequate and low available iron.

Variety	Dry Weight of Four Plants	
	Normal Soil (Adequate Available Iron)	Calcareous Soil (Low Available Iron)
Efficient		
1	8.48	3.97
2	8.69	4.24
3	10.90	3.54
4	10.26	4.24
Mean	9.58	4.00
Inefficient		
5	8.89	1.98
6	8.79	2.09
7	9.18	1.80
8	8.29	1.32
9	8.75	2.04
0	6.80	1.67
Mean	8.45	1.82

The analysis of variance of dry weights of tops from the two types of soil appears in Table 3.

Table 3. Analysis of variance of dry top weights of four efficient and six inefficient soybean varieties when grown in calcareous and normal soil.

Source of Variation	D F	Mean Squares	
		Calcareous Soil	Normal Soil
Reps.	2	0.07	6.89
Varieties:			
Eff. vs. Ineff.	1	34.35**	9.19
Within Eff.	3	0.33	4.20
Within Ineff.	5	.25	2.20
Error	18	.188	2.302

**F value exceeds the 1 percent point in level of significance.

The analysis of variance substantiates the visual differential performance of the efficient and inefficient varieties when grown in the low-available-iron calcareous soil as illustrated in Fig. 4. No significant difference in top yields existed between the two types when grown on the soil containing an adequate supply of available iron. As the moisture content of the two types did not differ significantly when grown on either soil, analysis of the green weights showed the same results.

The results in Table 2, although indicating a far greater growth by the efficient varieties than by the inefficient varieties on calcareous soil, also indicate a much greater growth on the normal soil than is exhibited by even the efficient varieties in the former medium. It is to be emphasized that the two soils in this study came from entirely different sources and undoubtedly the "normal" soil was much higher in availability of major growth elements as well as in available

iron. The only object in using it was to demonstrate the non-differential growth of the efficient and inefficient varieties in soils containing adequate available iron. The demonstration would have been more effective if calcareous soil in which iron was made available could have been used. When iron compounds were added to highly calcareous soil, however, the iron seemed to become non-available very rapidly, as chlorosis was not entirely eliminated in the inefficient varieties. Addition of acids corrected chlorosis readily, but such treatment was thought to induce other changes in the soil.

In a fourth replication of the above experiment, the "normal" soil was obtained approximately 30 feet distant from the highly calcareous area from which the alkaline soil was obtained. Although the soil from the adjacent source contained approximately three percent calcium carbonate, no chlorosis was observed in the inefficient varieties. In this replication the mean dry top yield of four plants of the efficient and inefficient varieties was, respectively, 2.97 and 1.55 gms. when grown in the highly calcareous soil, and 3.49 and 3.59 when grown in the low carbonate soil. These two soils were undoubtedly more nearly alike in regard to major growth elements than the two soils used in the above experiment.

Inheritance of Efficiency in Iron Utilization

Genotype classification in the inheritance studies was based on the degree of chlorosis exhibited by the plants when grown in a nutrient solution which had been rendered low in available iron. Maximum

chlorosis was determined during development of the first and second compound leaf. All plants were assigned grades ranging from 0 to 5 depending on the degree of chlorosis exhibited.

Although the testing of progenies extended throughout periods of low and adequate light intensities, as encountered throughout the winter and spring seasons, overlapping of classification of the efficient and inefficient parents occurred infrequently. Therefore, all tests of plants with comparable genetic constitution were grouped regardless of the period of testing.

Performance of parents

The performance of all parent plants, which were included in the various tests, has been summarized in Table 4.

Table 4. Summary of performance of efficient and inefficient parents when tested on low-available-iron media.

Variety	Chlorosis Grade						Mean Grade
	0	1	2	3	4	5	
Efficient:							
1	26	-	-	-	-	-	0.0
2	20	3	1	-	-	-	.2
3	30	2	-	-	-	-	.1
4	38	4	-	-	-	-	.1
Total	114	9	1	-	-	-	.09
Inefficient:							
5	-	-	3	1	8	19	4.4
6	-	-	3	7	9	2	3.5
7	-	-	-	-	6	9	4.6
8	-	2	3	20	19	25	3.9
9	-	4	9	18	24	15	3.5
0	-	2	7	8	7	6	3.3
Total	-	8	25	54	73	76	3.78

The average chlorosis grade assigned the efficient parents was 0.09 while the mean of the inefficient parents was 3.78. Only one plant of the efficient parents was assigned a chlorosis grade above 1 and only 8 inefficient parent plants were assigned a grade below 2. Consequently, if the genotypes of these plants had not been known, and if all plants assigned chlorosis grades of 0 and 1 would have been considered efficient genotypes, and plants assigned grades 2 to 5 inefficient genotypes, 9 plants out of 360, or 1 out of 40 would have been classified erroneously. The frequency of error in genotype classification in segregating progenies was probably of this same magnitude.

Performance of F_1 crosses

The frequency distribution of F_1 crosses within the six chlorosis grades in comparison with that of the parents is presented in Table 5. In this classification grades 0 to 1 included plants in which no definite iron chlorosis appeared and grades 2 to 5 included plants showing different degrees of iron deficiency symptoms. The frequency distributions have been grouped into these two major classes.

Table 5. Frequency distribution of chlorosis grades of parents and various F_1 crosses.

Genetic Material Studied	Chlorosis Grades							
	0	1	Total 0 to 1	2	3	4	5	Total 2 to 5
Eff. Parents	114	9	123	1	-	-	-	1
Ineff. Parents	-	8	8	25	54	73	76	228
Eff. x Eff. F_1s	44	3	47	-	-	-	-	0
Ineff. x Ineff. F_1s	-	1	1	6	14	23	86	129
Ineff. x Eff. F_1s	87	5	92	-	-	-	-	0

Very little overlapping in the classification of the two parental types occurred. In all six efficient x efficient crosses only efficient F_1 plants were obtained. The 13 F_1 crosses of inefficient x inefficient types resulted only in inefficient progeny, indicating that all the inefficient varieties possessed a common gene or genes conditioning the inefficiency in iron utilization. F_1 plants in each of 14 crosses between inefficient and efficient varieties were as efficient in iron utilization as their efficient parents, indicating complete dominance of this type. The dominance of iron efficiency under field conditions on calcareous soil is illustrated in Fig. 5.

Performance of efficient x inefficient F_2 populations

F_2 populations of all possible crosses between the four efficient and six inefficient parents were tested in a nutrient solution rendered low in available iron. The F_2 data appear in Table 6. The ratio of efficient to inefficient plants in the total for all F_2 populations does not deviate significantly from the ratio of a 3:1 hypothesis. Each cross also has been tested for conformity to this ratio by the chi-square test thereby revealing close agreement to a 3:1 ratio. The difference between the four efficient and six inefficient varieties in efficiency of iron utilization seems to be conditioned by a single major gene.

Fig. 5. Three plants of parent 8 (left), three 8 x 4 F_1 plants, and two plants of parent 4 when growing on calcareous soil.

Fig. 6. Three F_3 lines when grown in low-available-iron nutrient solution - homozygous inefficient (upper row), segregating (center row) and homozygous efficient (lower row).

Table 6. Numbers of efficient and inefficient plants in F_2 populations resulting from crosses between efficient and inefficient varieties when grown in media low in available iron.

Cross	Efficient	Inefficient	Chi-Square* (3:1)
1 x 5	45	14	.05
1 x 6	37	17	1.21
1 x 7	38	21	3.53
1 x 8	40	15	.15
1 x 9	41	12	.16
1 x 0	33	12	.07
2 x 5	38	16	0.65
2 x 6	38	15	.31
2 x 7	26	9	.01
2 x 8	38	14	.10
2 x 9	36	10	.26
2 x 0	43	10	1.06
3 x 5	40	15	0.15
3 x 6	44	10	1.21
3 x 7	43	14	.01
3 x 8	41	14	.01
3 x 9	131	39	.38
3 x 0	41	12	.16
4 x 5	21	8	0.10
4 x 6	30	12	.29
4 x 7	35	11	.03
4 x 8	26	13	1.44
4 x 9	38	15	.31
4 x 0	13	4	.02
Total all F_2s	866	305	0.69

*X^2 for 1 D F at 5 percent level = 3.84

Performance of backcross populations

F_1 plants from five crosses between two inefficient and three efficient parents were backcrossed to their respective inefficient parents. The frequency distributions of the backcross populations in regard to iron utilisation efficiency are presented in Table 7.

Table 7. Numbers of efficient and inefficient plants in backcross populations.

Backcross	Efficient	Inefficient	Chi-Square* (1:1)
8 x (8x1)	13	12	0.04
8 x (8x2)	32	29	.02
8 x (8x3)	7	13	1.80
Total X^2			1.86
Total Plants	52	54	.04
9 x (1x9)	24	22	0.09
9 x (3x9)	14	10	.067
Total X^2			.76
Total Plants	38	32	.51
Total	90	86	0.09

*X^2 for 1 D F at 5 percent level = 3.84
X^2 for 2 D F at 5 percent level = 5.99
X^2 for 3 D F at 5 percent level = 7.81

None of the backcross populations in Table 7 yield a ratio of efficient to inefficient plants differing significantly from a 1:1 ratio.

Performance of F_3 lines

During the summer of 1940 F_2 populations from efficient x inefficient crosses involving efficient parents 2 and 4, and inefficient parents 6, 8, 9, and O, were grown to maturity in soil with adequate available iron. The progeny of a limited number of F_2 plants, selected at random from each cross, were tested to determine their efficiency in iron utilization when grown in nutrient solution containing a low level of available iron. Because of limited facilities, only 10 F_3 plants were grown as a test of each F_2 individual. If a single factor difference is assumed, this small number of plants could result in erroneous classification of approximately seven percent of the segregating progenies. The F_3 progenies appear in Table 8. A segregating line grown by chance between apparently homozygous efficient and inefficient lines is shown in Fig. 6.

Table 8. Genetic classification of efficient x inefficient F_2 populations on the basis of their progeny performance in the F_3 generation when grown in media low in available iron.

Cross	Homozygous Efficient	Segregating	Homozygous Inefficient	Chi-Square* (1:2:1)
6 x 2	2	9	5	1.37
8 x 2	3	7	7	2.41
9 x 2	3	9	6	1.00
O x 2	3	8	5	.50
4 x 6	2	10	6	2.00
4 x 8	6	5	4	2.20
4 x 9	2	11	3	2.37
4 x O	3	10	5	.67
Total X^2				12.52
Total Plants	24	69	41	4.43

*X^2 for 2 D F at 5 percent level = 5.99
X^2 for 16 D F at 5 percent level = 26.30

The proportion of homozygous efficient, segregating, and homozygous inefficient F_3 lines in none of the F_2 populations deviates significantly from the expected 1:2:1 ratio. Furthermore, the sum of the chi-square values for all populations, and the chi-square value calculated from the deviations of total plants in each class from the expected number are also non-significant.

The numbers of efficient and inefficient plants in the segregating lines of each cross involved in the progeny test of F_2 plants are presented in Table 9.

Table 9. Numbers of efficient and inefficient plants in F_3 segregating lines.

Cross	Efficient	Inefficient	Chi-Square* (3:1)
6 x 2	66	26	0.52
8 x 2	47	19	.51
9 x 2	63	24	.31
0 x 2	56	23	.71
4 x 6	70	29	.97
4 x 8	34	16	1.31
4 x 9	79	31	.59
4 x 0	75	24	.03
Total X^2			4.95
Total Plants	490	192	3.61

* X^2 for 1 D F at 5 percent level = 3.84
X^2 for 8 D F at 5 percent level = 15.51

None of the population totals for segregating lines deviated significantly from the 3:1 ratio. The total chi-square, as well as the chi-square for the ratio of all efficient and inefficient plants in the segregating lines, also indicates conformity with the single factor hypothesis.

Maternal inheritance test

As reciprocal crosses between efficient and inefficient parents were frequently made, the data were examined to determine the possibility of maternal inheritance (Table 10).

Table 10. Performance of F_1 and F_2 generations of reciprocal crosses.

Type of Parent		Chlorosis Grades								Chi-Square*
Female	Male	0	1	Total Eff.	2	3	4	5	Total Ineff.	(3:1)
Ineff.	x Eff. F_1	60	3	63	-	-	-	-	0	-
Eff.	x Ineff. F_1	42	2	44	-	-	-	-	0	-
Ineff.	x Eff. F_2	302	45	347	14	48	33	38	133	1.74
Eff.	x Ineff. F_2	451	68	519	26	62	51	34	173	0.00

*X^2 for 1 D F at 5 percent level = 3.84

Absence of maternal inheritance is evidenced by the similarity in performance of F_1 and F_2 generations resulting from reciprocal crosses.

Physiological Study

Leaf and stem tissues of the four efficient and six inefficient varieties used in the inheritance study were subjected to certain chemical analyses. These varieties were grown in a nutrient solution tank containing adequate available iron for even the inefficient parents. Four plants of each variety were grown and harvested as a unit, such units being replicated four times.

The following analyses were made; (1) pH of composite tissue fluid expressed after freezing samples of leaves and stems, (2) soluble

iron in one ml. of composite tissue fluid from leaves and stems, (3) total iron in dried stem and leaf tissues, and (4) total potassium in dried leaf tissues. Mean values of the four replications for the four efficient and six inefficient varieties are presented in Table 11.

Table 11. Mean pH values of composite tissue fluids, soluble and total iron of leaf and stem tissues, and total potassium of leaf tissues of four efficient and six inefficient soybean varieties.

	Leaves				Stems		
	pH	Soluble Fe (ppm.)	Total Fe (mg.Fe/gm.D.M.)	Total K (%)	pH	Soluble Fe (ppm.)	Total Fe (mg.Fe/gm.D.M.)
Efficient:							
1	6.27	6.28	.978	3.56	5.90	5.70	.163
2	6.20	6.70	.963	3.75	5.88	6.10	.164
3	6.18	7.50	.935	3.90	5.87	5.08	.143
4	6.12	6.53	.848	4.04	5.83	6.45	.147
Mean	6.19	6.75	.928	3.81	5.87	5.83	.152
Inefficient:							
5	6.51	4.15	1.210	3.48	5.99	5.93	.186
6	6.43	4.98	1.103	3.48	5.98	5.00	.188
7	6.37	6.73	.975	3.70	5.96	6.10	.163
8	6.50	5.58	1.353	3.39	5.99	5.50	.171
9	6.37	6.20	1.005	3.58	5.92	6.15	.174
0	6.43	5.68	1.048	3.52	5.95	4.98	.181
Mean	6.43	5.68	1.112	3.52	5.97	5.61	.177

The criteria, for which means are presented in Table 11, have been subjected to analysis of variance (Table 12).

Table 12. Analysis of variance of pH, soluble iron, and total iron of leaf and stem tissues, and total potassium of leaf tissue of ten soybean varieties.

Source of Variation	D F	Mean Squares			
		pH	Soluble Iron	Total Iron	Total Potassium
Leaves:					
Replications	3	.0034	0.10	.0264	.1425
Varieties:					
Eff. and Ineff.	1	.5520**	11.10*	.3271**	.8109**
Within Efficient	3	.0164	1.12	.0126	.1638
Within Inefficient	5	.0151*	3.81	.0742**	.0438
Error	27	.00584	1.519	.01740	.09022
Stems:					
Replications	3	.0006	0.32	.0000	
Varieties:					
Eff. and Ineff.	1	.0920**	0.48	.00616**	
Within Efficient	3	.0039*	1.39	.00053	
Within Inefficient	5	.0028	1.14	.00038*	
Error	27	.00118	0.810	.000133	

*F value exceeds the 5 percent point in level of significance
**F value exceeds the 1 percent point in level of significance

The analysis of variance shows that efficient and inefficient varieties differed significantly in hydrogen ion concentration of composite tissue fluid of both leaves and stems. Although significant differences were found in the pH of leaf tissue among the inefficient, and in the pH of stems among the efficient varieties, these differences were far less in magnitude than differences between the efficient and inefficient varieties. The F values for leaves and stems of the latter were, respectively, 12 and 10 times the value necessary for highly significant differences. Practically the same result was obtained from

the total iron analyses of leaves and stems. The inefficient varieties, which had the higher pH of expressed sap, had a surprisingly greater amount of total iron in the tissues than that in the efficient varieties.

Analyses for soluble iron show a significantly larger quantity of iron in the expressed sap of leaf tissue from the efficient type. Although soluble iron in the stems shows the same relationship, the differences are statistically non-significant. Total potassium was significantly greater in the efficient varieties, while varieties within the two groups did not differ significantly.

The gene conditioning greater efficiency in iron utilization thus seems to be expressed by its action in producing higher hydrogen ion concentration, higher soluble iron, higher potassium and lower total iron content of aerial tissues than its allel which, when in the homozygous state, conditions inefficiency in iron utilization.

A study of the associations between these various compositional criteria was made by means of the covariance method of analysis (39). The relative degree of association was considered a possible means of approaching the cause and effect relationship. The following associations in the leaf tissues were investigated by means of covariance: pH and soluble iron, potassium and soluble iron, pH and total iron, potassium and total iron and pH and potassium. The stem analysis associations studied were pH and soluble iron, and pH and total iron. The resulting errors of estimate, mean squares, and adjusted mean values for the efficient and inefficient varieties appear in Table 13. Mean squares from the analysis of variance, and unadjusted mean values for the two types of varieties are included to facilitate comparison of adjusted and unadjusted values.

Table 13. Mean squares of errors of estimate and adjusted mean values of efficient and inefficient varieties resulting from the analysis of covariance of various compositional criteria.

Source of Variation	D F	Soluble Iron			Total Iron			Total K	
		Unadj.	Adj. for pH	Adj. for K	Unadj.	Adj. for pH	Adj. for K	Unadj.	Adj. for pH
Leaf Tissues:									
Varieties									
Eff. and Ineff.	1	11.10*	0.23	3.02	.3271**	.0247	.0798*	.8109**	.0024
Within Eff.	3	1.12	1.21	0.95	.0126	.0034	.0022	.1668	.0445
Within Ineff.	5	3.81	2.02	3.05	.0742**	.0168	.0475**	.0438	.0140
Error	27[1]	1.519	1.372	1.38	.01740	.0091	.0111	.09022	.08263
Variety Means:									
Efficient		6.75	5.91	6.49	.928	1.103	.975	3.81	3.62
Inefficient		5.68	6.24	5.85	1.112	.995	1.080	3.52	3.65
Difference		1.07	-0.33	0.64	-.184	.108	-.105	0.29	-0.03
Stem Tissues:									
Varieties									
Eff. and Ineff.	1	0.48	1.04		.00616**	.00013			
Within Eff.	3	1.39	0.78		.00050	.00014			
Within Ineff.	5	1.14	1.02		.00039*	.00039**			
Error	27[1]	0.810	0.743		.000133	.000097			
Variety Means:									
Efficient		5.83	5.30		.152	.162			
Inefficient		5.61	5.97		.177	.170			
Difference		0.22	-0.67		-.025	-.008			

*F value exceeds 5 percent point in level of significance
**F value exceeds 1 percent point in level of significance
[1]26 D F for mean squares of errors of estimate.

The mean squares of errors of estimate in Table 13 indicate a close association between the hydrogen ion concentration of the expressed juice and total iron within the leaf and stem tissues. Highly significant total iron differences between the efficient and inefficient varieties were rendered non-significant after due allowance was made for pH differences.

Similarly, potassium and soluble iron differences within leaf tissues became non-significant when pH variation was taken into account. Although adjustment for the potassium association did reduce soluble and total iron differences between the efficient and inefficient genotypes, the potassium content did not seem to account for as much of the iron variation as was explained by the hydrogen ion concentration differences.

DISCUSSION

In view of the complex nature of mineral absorption and utilization in plants, the monogenic simplicity of the genetic control of iron utilization efficiency discovered in these investigations seems remarkable. Although some variation of efficiency was noted among the inefficient varieties, the magnitude of expression of any modifying genes was negligible in comparison with that of the major gene involved.

As the gene conditioning inefficiency proved identical in the six varieties, and as fourteen additional inefficient varieties probably carried this gene, an effort was made to trace the origin of varieties of this type. These varieties came from central and southern Manchuria or northern Chosen. Several of the inefficient varieties were collected from the same locality but, in all cases, efficient varieties were also obtained from the same locality. No marked selection against the inefficient genotype seems to have occurred. This is especially odd since calcareous and alkaline soils of various kinds occur generally throughout central and southern Manchuria (42). As soybean varieties in the Orient are generally developed for local areas and normally do not spread rapidly, it is possible that the inefficient varieties were grown on upland soil from which lime had been leached, and no selection against this genotype occurred.

Chemical analyses of stem and leaf tissues were made to determine the physiological differentiation between the genotypes. Differences in hydrogen ion concentration of expressed tissue fluid were the most consistent of the constituents measured. Such differences were obtained not only when the two genotypes were grown in nutrient solutions but also when grown on normal soil under field conditions. The hydrogen ion concentration of expressed cell sap is open to criticism if the inference is made that the values obtained represent the true intracellular pH. As pointed out by Osterhout (30), expressed juice does not represent an unaltered cell sap, for in crushing the cells, the sap may be changed by chemical reactions, absorption, or admixture with intercellular substances. Rogers and Shive (33) found that although various tissues within a plant differed greatly in hydrogen ion concentration, the pH of expressed fluid was fairly consistent and approximated the mean of the hydrogen ion concentration of the various tissues. Furthermore, the pH of composite tissue fluid of various species was proportionate to the pH of similar tissues within the different species. The observed pH difference of the efficient and inefficient soybean varieties is therefore believed to be proportional to the intracellular pH difference of the two types.

Relatively lower solubility of the iron and lower potassium content was associated with the higher pH of the inefficient genotype. The interrelation of cell sap pH, total and soluble iron, and potassium content of plant tissues is not well understood although various associations have been reported. The investigations of Ingalls and Shive (18)

and Rogers and Shive (33) showed a definite relationship between the pH of expressed tissue fluid and the solubility and quantity of iron present within the plant. Hoffer and Trost (16) contended that excessive accumulations of iron in the nodal regions of the corn stem were often associated with inadequate supplies of available potassium in the growth medium, and consequently, in the corn stems. The interrelation of absorption of potassium and calcium by plants, as found by Loehwing (24), may be of significance. He also found that a consistent decrease of pH in the expressed plant sap was associated with the addition of potassium to the growth medium. This relationship was considered responsible for a lack of iron deficiency symptoms accompanying this treatment. An increase in the potassium concentration of cell saps could be expected to result in pH reduction if a bicarbonate-carbonic acid system is present as a buffering agent. That such a system is present in expressed sap and that such a buffering system is effective on the alkaline side of pH 5.8 was noted by Small (37) in Vicia faba. As the pH of expressed sap of soybeans is consistently above this pH it may well be that such a buffering system is present.

In view of these various findings it is suggested that the inefficient gene may condition lower potassium concentration which produces higher pH of cell sap and thereby causes the solubility of iron to be decreased. Although in this study potassium analyses were conducted as an afterthought when genotype differences in iron solubility were discovered, an investigation is planned to ascertain the comparable effi-

ciency of the two genotypes when grown on various levels of potassium. The results of such an experiment coupled with tissue analyses of iron and potassium should give further information on this complex cause-and-effect relationship.

SUMMARY

1. Marked differences in chlorosis were noted among soybean varieties when tested on calcareous soils for the first time since their introduction into the United States from Manchuria.

2. Testing of these varieties in nutrient solution cultures and in sub-irrigated crushed quartz media proved that this differential performance could be induced when the plants were grown on media in which the concentration of available iron was low. When grown on such media, varieties which were efficient in iron utilization made normal, green growth, while inefficient varieties showed severe chlorosis symptomatic of iron deficiency which ultimately resulted in death of the plant.

3. As the difference between efficient and inefficient genotypes was of sufficient magnitude to permit classification when grown in media rendered low in available iron, the mode of inheritance of efficiency differences was determined.

4. On the basis of F_2 and F_3 populations of crosses between four efficient and six inefficient varieties, and backcross populations, differences in efficiency of iron utilization were shown to be conditioned by a single gene.

5. Inefficiency of F_1 plants from all crosses among the six inefficient varieties established allelomorphism of the gene conditioning inefficiency, and the assumption was made that the inefficient gene is identical in these varieties.

6. Performance of F_1 plants from crosses between efficient and inefficient varieties indicated complete dominance of the efficient allel.

7. Absence of maternal inheritance was evidenced by similarity in performance of F_1 and F_2 generations from reciprocal crosses between the efficient and inefficient types.

8. Composition in aerial plant tissues, as conditioned by the inefficient gene, consisted of relatively higher pH, lower soluble iron, higher total iron, and lower potassium content. Although the data furnished no definite information as to identity of the primary causal agent, the assumption was made that relatively low solubility of iron in inefficient genotypes was induced by the comparatively high pH. On the other hand, relatively low potassium content might have accounted for the low hydrogen ion concentration.

ACKNOWLEDGEMENTS

The writer wishes to express his appreciation to Drs. E. W. Lindstrom and I. J. Johnson for helpful suggestions and criticism during the course of these investigations.

Thanks are also extended to Mr. W. J. Morse, Washington, D. C., for information concerning the source of inefficient varieties.

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